Thrombelastography is Better Than PT, aPTT, and Activated Clotting Time in Detecting Clinically Relevant Clotting Abnormalities After Hypothermia, Hemorrhagic Shock and Resuscitation in Pigs

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Background: **Hypothermia and hemorrhagic shock contribute to coagulopathy after trauma. In this study, we investigated the independent and combined effects of hypothermia and hemorrhage with resuscitation on coagulation in swine and evaluated clinically relevant tests of coagulation.**

Methods: **Pigs (n = 24) were randomized into four groups of six animals each: sham control, hypothermia, hemorrhage with resuscitation, and hypothermia, hemorrhage with resuscitation combined. Hypothermia to 32°C was induced with a cold blanket. Hemorrhage was induced by bleeding 35% of total blood volume followed by resuscitation with lactated Ringer's solution. Coagulation was assessed by thrombin generation, prothrombin time (PT), activated partial thromboplastin time (aPTT), activated clotting time (ACT), and thrombelastography (TEG) from blood samples taken at baseline and 4 hour after hypothermia and/or hemorrhage with resuscitation. Data were compared with analysis of variance.**

Results: **Baseline values were similar among groups. There were no changes in any measurements in the control group. Compared with baseline values, hemorrhage with resuscitation increased lactate to 140% ± 15% ($p < 0.05$). Hypothermia decreased platelets to 73% ± 3% ($p < 0.05$) with no effect on fibrinogen. Hemorrhage with resuscitation reduced platelets to 72% ± 4% and fibrinogen to 71% ± 3% (both $p < 0.05$), with similar decreases in platelets and fibrinogen observed in the combined group. Thrombin generation was decreased to 75% ± 4% in hypothermia, 67% ± 6% in hemorrhage with resuscitation, and 75% ± 10% in the combined group (all $p < 0.05$). There were no significant changes in PT or aPTT by hemorrhage or hypothermia. ACT was prolonged to 122% ± 1% in hypothermia, 111% ± 4% in hemorrhage with resuscitation, and 127% ± 3% in the combined group (all $p < 0.05$). Hypothermia prolonged the initial clotting time (R) and clot formation time (K), and decreased clotting rapidity (α) (all $p < 0.05$). Hemorrhage with resuscitation only decreased clot strength (maximum amplitude [MA], $p < 0.05$). TEG parameters in the combined group reflected the abnormal R, K, MA, and α observed in the other groups.**

Conclusion: **Hypothermia inhibited clotting times and clotting rate, whereas hemorrhage impaired clot strength. Combining hypothermia with hemorrhage impaired all these clotting parameters. PT, aPTT were not sensitive whereas ACT was not specific in detecting these coagulation defects. Only TEG differentiated mechanism related to clotting abnormalities, and thus may allow focused treatment of clotting alterations associated with hypothermia and hemorrhagic shock.**

Key Words: **Thrombin, Coagulation, Thrombelastograph, Blood clotting tests, Hemorrhage.**

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Hemorrhage is the leading potentially preventable cause of death on the battlefields and a major cause of death in civilian trauma.[1,2] In addition to functional alterations in heart, liver, lung, kidney, and gastrointestinal systems,[3–5] hemorrhage disrupts the coagulation process, resulting in the clinical consequences of uncontrolled bleeding, disseminated intravascular coagulation, and thrombotic complications.[2,5–10] Although hemorrhage-associated coagulation complications have been demonstrated in trauma patients,[8–10] our understanding of these coagulation abnormalities is limited to the depletions of coagulation components and factors.

Deliberate hypothermia is being used as a neuroprotective mechanism during cardiopulmonary bypass surgery.[11,12] However, accidental hypothermia is associated with abnormal bleeding and increased mortality in trauma victims.[13–17] Hypothermia of 32°C alone is associated with a 23% mortality rate; less than 32°C of trauma induced-hypothermia is associated with nearly 100% mortality as a result of arrhythmias.[13,14,16] The effects of hypothermia on coagulation relate to inhibition of platelet function and reduced clot-

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ting factor activities.[18–25] However, the combined effect from hemorrhagic shock and hypothermia on the coagulation process has received little attention. In addition, clinical standard coagulation tests (i.e., PT, aPTT) are often normal despite significant changes in coagulation function[19,21,26] and comparisons of these tests are infrequent.

The purpose of this study was to investigate the individual and combined effects of hypothermia and hemorrhage on the clotting process in a swine model. Coagulation abnormalities were characterized by changes in platelet counts, fibrinogen levels, thrombin generation, and clotting function evaluated by standard clinical tests of PT, aPTT, activated clotting time (ACT), and thrombelastography (TEG) after hemorrhagic shock and hypothermia.

METHODS

This study was approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical Research and adhered to the NIH guidelines for the Care and Use of Laboratory Animals (DHHS Publication NIH 88-23). A total of 24 pigs (39 ± 1 kg) were randomly assigned into four groups: the sham control group (control, n = 6), the hemorrhage-resuscitation group (hemorrhage, n = 6), the hypothermia group (hypothermia, n = 6), and the hemorrhage-resuscitation hypothermia combined group (combined, n = 6). After an overnight fast, animals were preanesthetized with glycopyrrolate (0.1 mg/kg) and telazol (6 mg/kg). The pigs were then intubated and maintained by 1.0%–1.5% isoflurane in 100% oxygen by mask for the surgical procedures. Polyvinyl chloride catheters were inserted into the thoracic aorta via the carotid artery for measurement of mean arterial pressures, heart rates, and temperatures. The right femoral artery was cannulated for arterial blood sampling and the left femoral artery for induction of bleeding. The left femoral vein was cannulated for lactated Ringer's (LR) resuscitation. The right femoral vein was cannulated for intravenous anesthesia of ketamine during the study.

Upon completion of catheter cannulation, anesthesia was switched to a combination of isoflurane (0.5%) and continuous intravenous drip of ketamine (0.15 mL/kg/h of 100 mg/mL) in all pigs for the remainder of the study period. After a 10-minutes stabilization period, blood samples were taken for baseline measurements (0 hour, normal pig body temperature of 39°C). Hemorrhagic shock was then induced in the hemorrhage group by bleeding approximately 35% of total blood volume (24.5 ± 0.1 mL/kg) over about a 30 minutes period from the left femoral artery to a preweighed canister on a balance. The rate of bleeding was controlled by adjusting the clamp on the left femoral artery catheter to maintain mean arterial pressure above 40 mm Hg. Afterward, pigs were resuscitated with LR at three times the bled volume over approximately 30 minutes. Pigs in the control and hypothermia groups were not bled or resuscitated. Hypothermia of 32°C was induced over approximately 1 hour in the hypothermia group using a cold blanket with 4°C circulating water. In the combined group, hemorrhagic shock and LR resuscitation were induced the same way as in the hemorrhage group and hypothermia was induced after starting of the 35% bleed. At 4 hours after baseline measurements (4 hour), blood samples were taken for measurements of coagulation and hemodynamics. Animals were euthanized afterward with an overdose of a veterinary euthanasia solution (FatalPlus, Fort Dodge, IA).

Mean arterial pressures and heart rate were recorded hourly during the study. Pig blood temperatures were monitored in vivo using intra-arterial sensors precalibrated according to the manufactures instructions (Paratrend 7 Trendcare System, Diametrics Medical, Roseville, MN). Cardiac output was measured hourly by thermodilution in triplicate during the study.

Analytical Methods

Platelet counts were measured from citrated blood using an ABX Pentra 120 Hematology Analyzer (ABX Diagnostics, Irvine, CA). Blood gas measurements (i.e., lactate) were analyzed by the Omni-9 Blood Gas Analyzer (AVL, Montpellier, France). Blood chemistries (i.e., total protein and albumin) were measured by the Dimension Clinical Chemistry System (Dade Behring, Newark, DE). Plasma fibrinogen concentration was measured using the BCS Coagulation System (Dade Behring, Deerfield, IL). PT and aPTT were measured using the BCS coagulation system at the pig's body temperatures when blood samples were taken (39°C (normal temperature for swine) or 32°C). ACT was measured in fresh whole blood using Hemochrone (HRFTCA 510 Hemochron, Internation Technique, Edison, NJ). TEG (TEG 5000 Hemostsis Analyzer, Hemoscope, Niles, IL) was performed using fresh whole blood samples with tissue factor at pig body temperatures (39°C or 32°C).

Thrombin generation was assessed by quantifying thrombin-antithrombin III (TAT) complex from minimally altered fresh whole blood samples, after the procedure described by Rand et al.[27] Briefly, fresh whole blood samples were added into tubes set on a shaker plate. After 20 minutes, a "quench" solution of 50 mmol/L EDTA and 10 mmol/L benzamidine in N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES)-buffered saline was added to the blood samples to stop clot formation. The quenched samples were centrifuged and supernatants were collected for TAT concentration measurement using commercially available enzyme-linked immunosorbent assay kits (Enzygnost TAT, Dade Behring, Deerfield, IL). The TAT concentrations from the supernatant samples reflect thrombin content generated from fresh whole blood samples before the addition of quench solution.

Statistical Analysis

Data were expressed as means ± SEM and analyzed using SAS statistical software. In each group, comparisons were made in all measurements on a pre/post basis using one-way analysis of variance. Between group comparisons were made with ap-

propriate adjustments for multiplicity using Tukey adjustment. The statistically significant level was set at $p < 0.05$.

RESULTS

Physiology and Hemodynamics

All animals survived to the end of the study. There were no significant changes observed in any measurements in the control group during the study. Mean arterial pressure was decreased in the hemorrhage, hypothermia, and the combined groups (Fig. 1). Cardiac output was initially elevated in the hemorrhage group at the 1-hour time period, followed by a return to baseline values (Fig. 1). In contrast, cardiac output only decreased compared with baseline in both the hypothermia and the combined groups

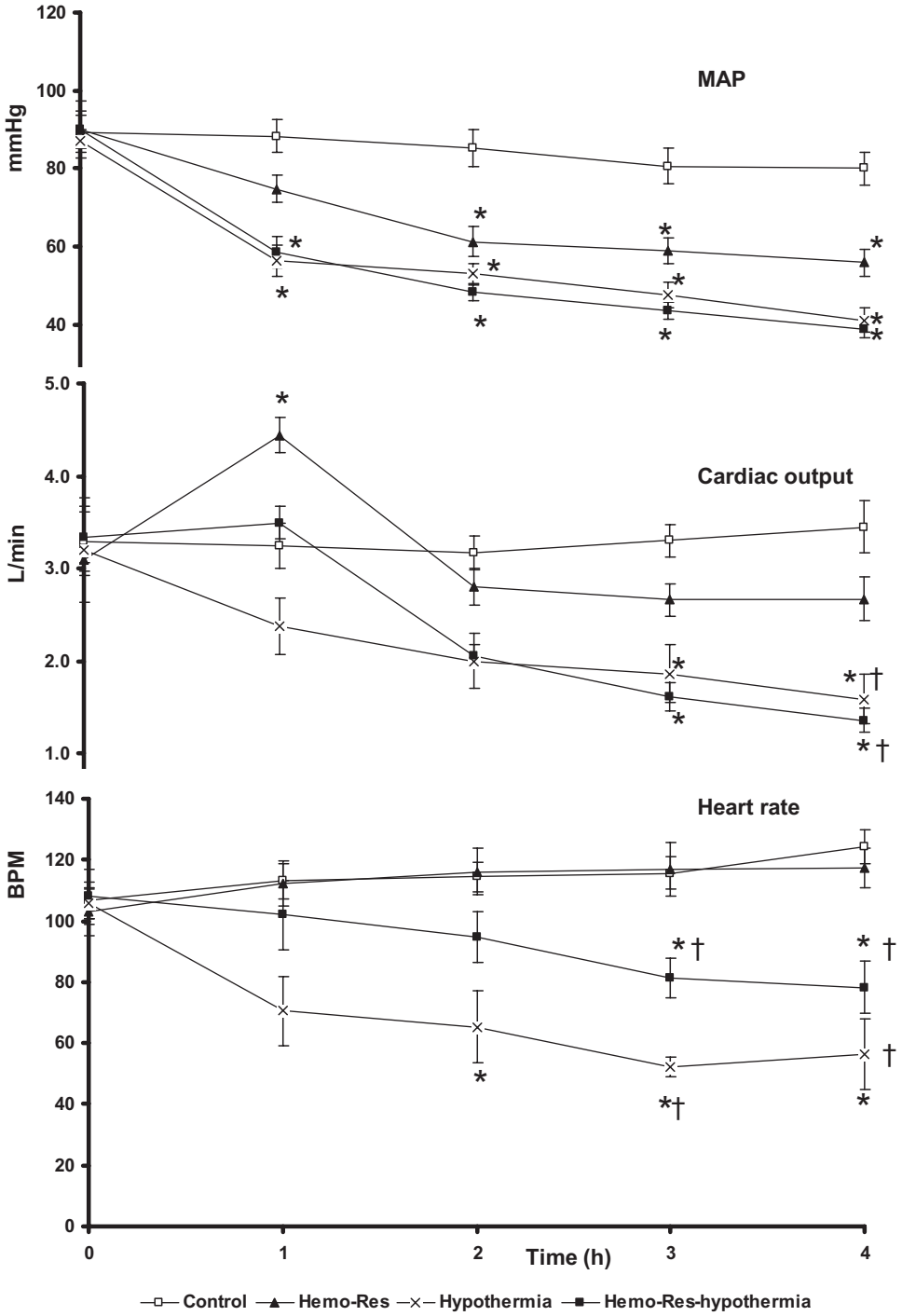

Fig. 1. *Physiologic changes after hypothermia and hemorrhage in pigs. Data represent mean ± SEM for six animals per group.* *p < 0.05 *compared with corresponding baseline.* †p < 0.05 *compared with changes in the hemorrhage group.*

(Fig. 1). Heart rate was decreased from baseline in the hypothermia and the combined groups, but not in the hemorrhage group (Fig. 1).

Compared with corresponding baseline values, lactate levels were increased similarly in the hemorrhage group (from 1.8 ± 0.1 mmol/L to 2.5 ± 0.2 mmol/L) and the hypothermia group (from 1.7 ± 0.4 mmol/L to 2.8 ± 0.4 mmol/L, both $p < 0.05$). A larger increase of lactate was observed in the combined group (from 1.8 ± 0.1 mmol/L to 7.1 ± 1.4 mmol/L, $p < 0.05$ compared with baseline and to the changes in the hemorrhage and hypothermia groups). Plasma total protein was similarly decreased in the hemorrhage group (from 5.5 ± 0.1 g/dL to 3.8 ± 0.1 g/dL) and the combined group (5.2 ± 0.3 g/dL to 3.3 ± 0.2 g/dL, both $p < 0.05$). Plasma albumin level was also similarly decreased in the hemorrhage group (65% ± 3% of baseline) and in the combined group (58% ± 4% of baseline, both $p < 0.05$). There were no significant changes in total protein and albumin in the hypothermia group.

Fibrinogen, Platelets, and Thrombin Generation

Plasma fibrinogen concentrations were similarly decreased in the hemorrhage group to 71% ± 3% of baseline and in the combined group to 65% ± 4% of baseline (both $p < 0.05$), with no significant changes in the hypothermia group (Fig. 2). Platelet counts were similarly decreased in the

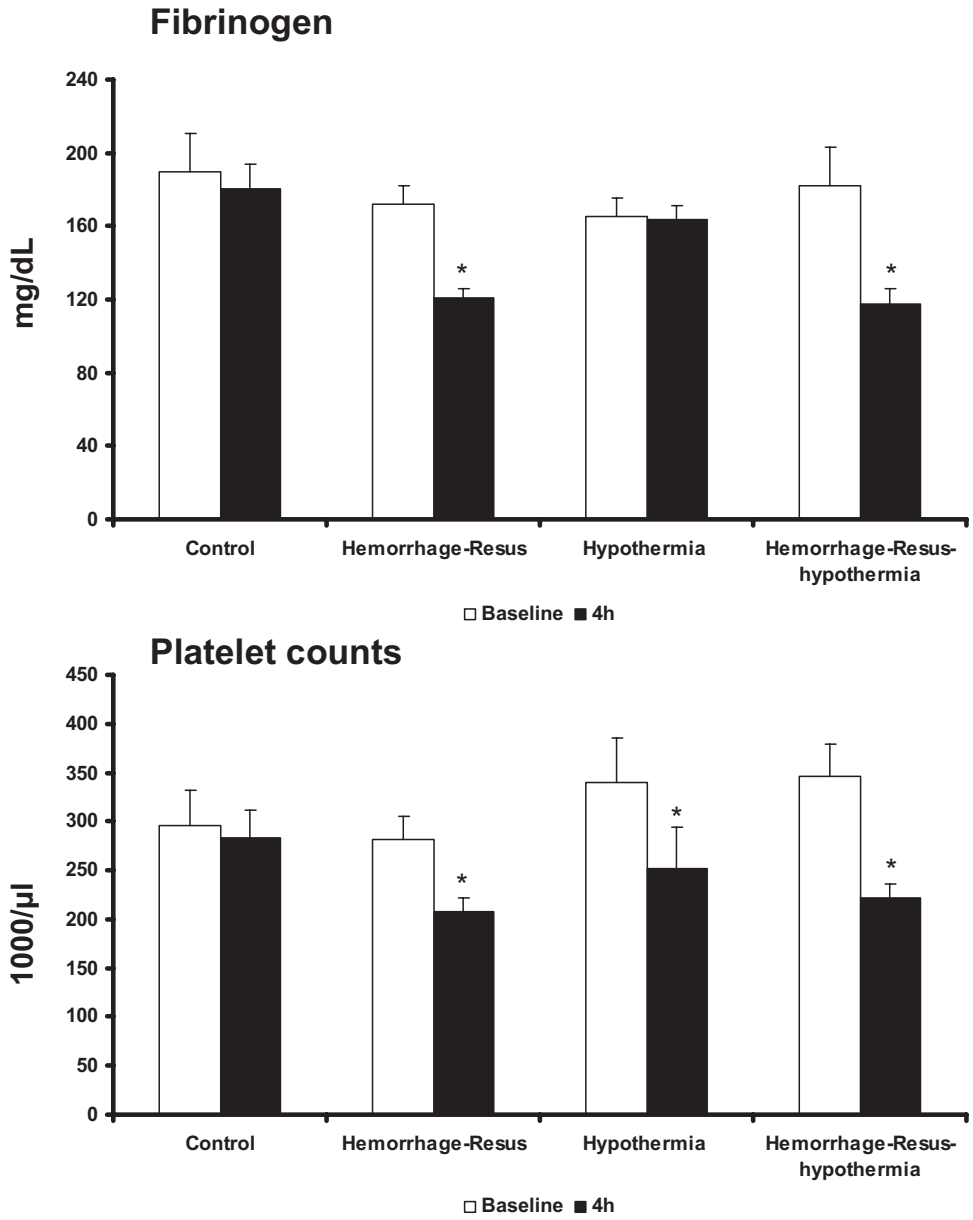

Fig. 2. *Changes in fibrinogen and platelet counts after hypothermia and hemorrhage with resuscitation in pigs. Data represent mean ± SEM for six animals per group.* *p < 0.05 *compared with corresponding baseline.*

Table 1 Changes in PT and aPTT After Hypothermia and/or Hemorrhage With Resuscitation

	PT (sec)			PTT (sec)		
	Baseline (39°C)	4 h (39°C)	4 h (32°C)	Baseline (39°C)	4 h (39°C)	4 h (32°C)
Control	10.1 ± 0.2	10.1 ± 0.1	N/A	16.0 ± 0.2	16.4 ± 0.2	N/A
Hemorrhage-resuscitation	10.3 ± 0.4	10.8 ± 0.4	N/A	15.9 ± 0.2	16.3 ± 0.2	N/A
Hypothermia	10.4 ± 0.2	10.3 ± 0.1	10.6 ± 0.2	15.2 ± 0.1	15.4 ± 0.2	15.9 ± 0.3
Combined*	10.3 ± 0.2	10.8 ± 0.3	14.0 ± 0.4†	15.9 ± 0.2	15.8 ± 0.2	16.3 ± 0.2

Data are means ± SE. Temperatures in parentheses were temperatures when assay was performed.
* $p < 0.05$ compared with baseline values.
† Combined is the hypothermia, hemorrhage with resuscitation combined group.
N/A indicates not applicable.

hemorrhage group, hypothermia and combined groups to 72% ± 4%, 73% ± 3%, and 66% ± 4% of baseline, respectively (all $p < 0.05$, Fig. 2). Thrombin generation, measured from fresh whole blood samples, was similarly decreased in the three experimental groups. Thrombin generation at 4 hour decreased to 67% ± 6% in the hemorrhage group, to 75% ± 4% in the hypothermia group, and to 75% ± 10% of baseline in the combined groups (all $p < 0.05$ compared with baseline).

Coagulation Tests

PT and aPTT were measured at pig body temperatures when blood samples were taken at baseline and at 4 hour during the study. In the hypothermia and the combined groups, PT and aPTT at 4 hour were also measured at 39°C and the pig target body temperature of 32°C for comparison. There were no changes in PT or aPTT observed in the hemorrhage group (Table 1). There were also no changes observed in PT or aPTT in the hypothermia group, regardless of the assay temperature (Table 1). In the combined group, PT was prolonged by 40% whereas aPTT remained unchanged when the assay temperature was 32°C (Table 1). However, when assayed at 39°C, there were no significant changes in PT or aPTT in the combined group (Table 1).

ACT measured from fresh whole blood was prolonged in the three experimental groups. ACT was increased from a baseline of 107 ± 6 seconds to 117 ± 2 seconds in the hemorrhage group. There was a larger increase in ACT in the hypothermia group (from baseline of 104 ± 2 seconds to 128 ± 3 seconds, $p < 0.05$ compared with baseline and to changes in the hemorrhage group). The increase of ACT in the combined group (from baseline of 105 ± 3 seconds to 133 ± 3 seconds, $p < 0.05$) was similar to that in the hypothermia group.

Coagulation function was assessed using TEG from fresh whole blood samples taken at baseline and 4 hour during the study. Hypothermia prolonged the initial clot formation time (*R* time) and time to maximum clot formation (*K* time), and slowed clot rapidity (α) with no effects on clot strength (MA, Fig. 3). In contrast, hemorrhage and resuscitation did not change *R* time, *K* time, or clot rapidity, but impaired clot strength. In the combined group, *R* time, *K* time, clot rapidity, and clot strength were all impaired (Fig. 3). Thus, there were independent additive effects on TEG measurements in the combined group.

DISCUSSION

In this study, we investigated the individual and combined effects of hemorrhage and hypothermia on the coagulation process in a swine model. The contribution of hypothermia and hemorrhage to coagulation was examined through clinical coagulation tests such as PT, and aPTT, fibrinogen levels, platelet counts, thrombin generation, and clotting function. Our data showed that hypothermia and hemorrhage affected different aspects of the coagulation process and both caused coagulation functional abnormalities. Further, the standard laboratory tests most clinicians use (i.e., PT and aPTT) to evaluate clotting function in trauma patients were inadequate to document these significant changes.

The coagulation process consists of a cascade of enzymatic reactions. The effect of temperature on an enzyme-catalyzed reaction is known as the Q_{10} effect, as the activity of an enzyme reaction is decreased by about 50% for every 10°C drop in temperature.[28] The clinical aPTT test represents enzymatic reactions in the intrinsic system, including the activation of factor XIIa, XIa, IXa, Xa, and IIa. In the current study, hypothermia of 32°C induced in vivo did not cause significant changes in aPTT (when assayed at 32°C or 39°C). This indicates that the drop in temperature from 39°C to 32°C did not affect the overall enzymatic activity in the intrinsic pathway. Similar observations were reported by Wolberg et al. in an in vitro study.[24] When plasma samples were chilled from 37°C to 33°C, the authors reported that there were no changes in aPTT.[23] It appears that the Q_{10} effect of a single enzyme reaction does not translate into a multienzyme reaction system in the coagulation process.

The clinical PT test reflects the enzymatic activation in the extrinsic system, which includes the activation of factor VIIa, Xa, and IIa. Similar to aPTT, PT did not change after hypothermia in this study (when assayed at 39°C or 32°C). When plasma samples from normal humans and from non-coagupathic patients were chilled from 37°C to 25°C, Reed et al.[20] and Gubler et al.[25] showed that PT and PTT were significantly prolonged by hypothermia less than 35°C. In the

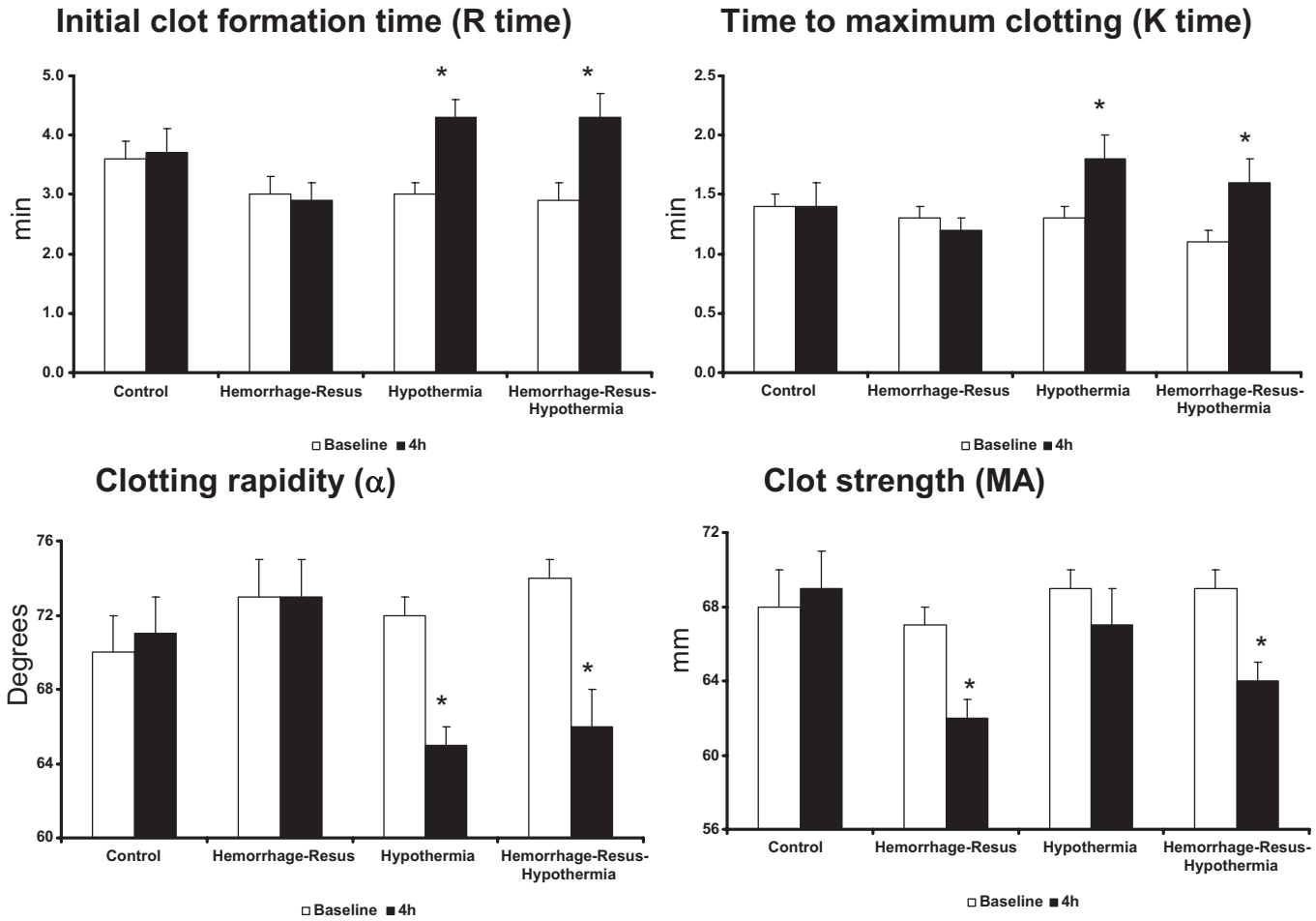

Fig. 3. *Changes in TEG measurements after hypothermia and hemorrhage with resuscitation in pigs. Data represent mean ± SEM for six animals per group.* *$p < 0.05$ *compared with corresponding baseline.*

present study, hypothermia of 32°C was induced in pigs and we did not observed significant changes in PT or aPTT (assayed at 32°C). The differences between our study and those studies may be due to the differences of hypothermia induced in a test tube versus in vivo, as well as differences in species. In addition, when hypothermia was combined with hemorrhage and resuscitation, PT (assayed at 32°C) was prolonged nearly 40%. Apparently both temperature drop and shock with hemodilution were necessary to cause a detectable change in PT. However, with faster and more severe hemorrhage, shock and hemodilution alone may possibly cause detectable changes in PT. It is worth mentioning that the changes in PT by combined hypothermia and hemorrhage were not detected when PT was assayed at 39°C, the pig's normal temperature, supporting the current clinical recommendation that advocates PT and aPTT testing at temperatures equivalent to the patient's body temperature.

Although PT (assayed at 32°C, pig body temperature when blood sample were withdrawn) was prolonged in the combined group, we did not observe significant changes in aPTT (assayed at 32°C) in the same group in this study. The lack of changes in aPTT and significant changes in PT in the combined group indicate that temperature has differential effects on the intrinsic and extrinsic pathways of the coagulation process. Further, since there are more enzymatic steps involved in aPTT when compared with those in PT, it is clear that the effects of temperatures on coagulation pathways are not simply an accumulation of effects from individual steps.

Besides temperature, enzyme concentrations contribute to the activities of enzyme reactions. In this study, a 35% blood loss and fluid resuscitation caused similar decreases in fibrinogen levels and platelet counts as the combined group. However, these decreases did not cause significant changes in PT or aPTT in this study. It is known that there are abundant coagulation components and factors present in the circulation. Thus, a 35% blood loss and fluid resuscitation in this study might not reduce the levels of coagulation factors below the threshold for normal function. Consistently, to correlate clotting factor deficiency to functional changes, Al Dieri et al.[29] measured changes of clotting potential (using thrombin potential measured by the area under the curve from the thrombogram) from plasma samples taken from patients with congenital deficiencies of various coagulation factors. The authors concluded that clotting factors had to be de-

creased to a considerable extent for changes of clotting potential to be detected. For example, factor V, VII, XI ,and X had to be decreased to 1% to 5% of normal values to cause a 50% change in clotting potential.[29] Thus, the simple moderate hemorrhage and LR resuscitation model used in this study did not deplete coagulation substances low enough to change clotting time (enzyme activities). However, a different situation might be expected after multiple injuries with uncontrolled hemorrhage in patients with severe trauma.

Platelet dysfunction from hypothermia has been reported in the literature.[18,22,23,24,30] In this study, hypothermia of 32°C caused a 28% drop in platelet counts, similar to the drop from blood loss and fluid resuscitation in the hemorrhage group (25%). The mechanisms leading to the decrease by hypothermia is unclear. When platelets isolated from human blood were chilled from 37°C to 33°C, Wolberg et al.[24] reported that platelet aggregation and adhesion were reduced. Thus, changes in aggregation and adhesion might possibly lead to an accelerated removal of platelet from the circulation and a decrease in platelet counts. Fibrinogen levels, in contrast, were not changed significantly from baseline by hypothermia in this study, while there was about a 30% depletion of fibrinogen levels observed in the hemorrhage and the combined groups. Although fibrinogen level was reduced by 30% after hemorrhage and resuscitation, there were no significant changes in TEG α (clotting rapidity) measurements. The lack of changes in α indicates that a larger drop in fibrinogen may be needed to cause significant changes in α, or fibrinogen may not be the only factor to affect α values. Considering a 35% blood loss induced in hemorrhage and combined groups and similar 30% to 35% drops in total protein and albumin were also observed in both groups, the depletion of fibrinogen in both groups most likely reflect the blood loss and fluid resuscitation-induced changes in blood volume.

Catalyzing the conversion of fibrinogen to fibrin clots, thrombin plays a central role in the clotting process.[31] Thrombin is generated from prothrombin upon the activation of factor Xa, Va, and platelets. In this study, hypothermia caused about a 25% decrease in thrombin generation. Similar decreases were observed in the hemorrhage and combined groups. Because platelet counts were similarly decreased in all three groups, we speculate that the inhibition in thrombin generation may be due to inhibition of platelet activation involved in thrombin generation process. Further investigation into this mechanism appears warranted.

ACT is a bedside clotting test that is often used for monitoring high-dose heparin anticoagulation. It measures the clot formation time from fresh whole blood samples in a tube containing an activator in the intrinsic pathway of coagulation.[32] ACT is thus similar to aPTT except that it includes the interaction of platelets with other clotting components. In contrast to aPTT, ACT in this study was prolonged by hypothermia and hemorrhage with fluid resuscitation, individually and combined. The differences between changes of ACT and aPTT suggest that platelets, which are not included in aPTT test, are likely an important contributor to changes observed in this study. In addition, since ACT was prolonged more in the hypothermia group (about 20%) than that in the hemorrhage group (about 10%), it is reasonable to speculate that hypothermia possibly cause more platelet dysfunction than a moderate blood loss and fluid resuscitation. Further, ACT was similarly prolonged by hypothermia and by hypothermia and hemorrhage with resuscitation combined in this study, supporting that hypothermia is possibly the primary contributor to the changes in ACT after hypothermia and hemorrhage.

The coagulation process is a symphony of inseparable interactions of fibrinogen, platelets, clotting enzymes, clotting factors, Ca^{++}, and the endothelium. The overall effects from individual factors are best presented in coagulation functional assessment. TEG provides comprehensive clotting profile analysis as it measures initial clot formation time, time to maximum clot, clot speed, clot strength, and fibrinolysis. In this study, TEG analysis showed that hypothermia delayed initial clot formation and decreased clot rapidity, but did not affect clot strength. Hemorrhage and resuscitation, on the other hand, did not change the initial clot formation or clot rapidity, but impaired clot strength. Thus, hypothermia and hemorrhage have differential effects on coagulation function. Since overall enzyme activity in this study did not appear to be a significant contributor, these functional changes are likely due to platelet dysfunction from hypothermia and depletion of fibrinogen and platelets from hemorrhage and fluid resuscitation. When hypothermia and hemorrhage with fluid resuscitation were combined, there were impairments in all of the above parameters, indicating independent additive effects. Our data suggest that comprehensive approaches (i.e., rewarming, supplementation of clotting factors, avoiding iatrogenic dilution, etc.) may be needed for effective treatments of trauma patients with hypothermia and hemorrhage associated coagulopathy.

Commonly used clinical coagulation tests include PT, aPTT, and ACT. Each of these tests monitors different aspects of coagulation. PT and aPTT are performed in platelet poor plasma, which exclude the interaction of platelets and other blood components. PT and aPTT reflect the overall activity from plasma clotting factors involved in the extrinsic and intrinsic pathways, respectively. Because the overall enzyme activity from extrinsic or intrinsic pathway did not appear to change significantly, and the significant contributor, platelets, was excluded in the measurements, PT or aPTT is therefore unable to adequately reflect coagulation defects from hypothermia and hemorrhage in this study. Indeed, Tripodi et al.[33] described that our current standard tests of PT and aPTT are useful to reveal information on a clotting process that exists in an in vitro test tube, but bears little relation to the in vivo perturbation of the coagulation system. On the other hand, significant changes in PT or aPTT indicate significant inhibition in the overall enzyme activities and

severe impairment in the enzymatic reaction driven coagulation process, as demonstrated in recent articles about PT or international normalized ratio (INR) being indicative of poor clinical outcomes.[34–38] Thus, although the insensitivity of PT or aPTT under some circumstances is not appreciated, it should be recognized that significant changes in PT and aPTT signal severe impairment in coagulation. As mentioned, ACT measures the clot formation time from fresh whole blood samples.[32] Since platelets and other blood clotting components are included, ACT is more sensitive than PT and aPTT in detecting clotting changes, as has been shown in this study. However, since it only monitors the clot formation time, ACT cannot specifically indicate changes in clotting speed and strength other than the clotting time. In contrast, TEG provides assessments of the initial clotting time, time to maximum clotting, clotting rapidity, clot strength, and fibrinolysis. Hypothermia and hemorrhage used in this study caused changes in these parameters, which have been reflected in TEG measurements in this study. Thus, the advantages of TEG measurements come from its comprehensive coverage of the coagulation process in helping to elucidate mechanisms associated with coagulation abnormalities associated with clinical conditions relevant to trauma.

TEG measurements with tissue factor provide coagulation changes through the activation of extrinsic pathway, whereas the measurements with kaolin reflect changes through the activation of intrinsic pathway. After trauma injury, tissue factor is released into blood stream to affect coagulation. Thus, coagulation changes measured through the activation of extrinsic pathway may be more physiologically relevant. Therefore, tissue factor was used in TEG measurements in this study. Since some TEG measurements are performed with kaolin in hospital setting, it is worthwhile to compare the two sets of measurements in our future studies.

In summary, we examined the systematic effects of hypothermia and hemorrhage on the coagulation process in a swine model. The overall enzyme activities in the extrinsic or intrinsic pathways, as reflected in the PT and aPTT tests, were not affected by hypothermia or hemorrhage, but platelets and thrombin generation were inhibited by hypothermia and/or hemorrhage with fluid resuscitation. Hypothermia and hemorrhage have differential effects on the coagulation process. Hypothermia inhibited the initial clot formation and clot rapidity, whereas hemorrhage and fluid resuscitation primarily impaired clot strength. Combined hypothermia and hemorrhage impaired all of these parameters. Compared with PT, aPTT, and ACT, TEG appears to be a better test to detect mechanisms associated with coagulation abnormalities induced by hypothermia and hemorrhage and may provide a guide for more focused treatments of clotting abnormalities after trauma.

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REFERENCES

1. Bellamy RF. The causes of death in conventional land warfare: implications for combat casualty care research. *Mil Med.* 1984; 149:55–62.
2. Sherman LA. DIC in massive transfusion. *Prog Clin Biol Res.* 1982; 108:171–189.
3. Collins JA. The pathophysiology of hemorrhagic shock. *Prog Clin Biol Res.* 1982;108:5–29.
4. Collins JA, Simmons RL, James PM, Bredenberg CE, Anderson RW, Heisterkamp CA III. Acid-base status of seriously wounded combat casualties. II. Resuscitation with stored blood. *Ann Surg*. 1971;173:6–18.
5. Hardy JF, De Moerloose P, Samama M. Massive transfusion and coagulopathy: pathophysiology and implications for clinical management. *Can J Anaesth*. 2004;51:293–310.
6. Armand R, Hess JR. Treating coagulopathy in trauma patients. *Transfus Med Rev*. 2003;17:223–231.
7. Sherry S. Hemostatic mechanisms and proteolysis in shock. *Fed Proc*. 1961;20:209–218.
8. Brohi K, Cohen MJ, Ganter MT, Matthay MA, Mackersie RC, Pittet JF. Acute traumatic coagulopathy: initiated by hypoperfusion: modulated through the protein C pathway? *Ann Surg*. 2007;245: 812–818.
9. Holcomb JB, McMullin NR, Pearse L, et al. Causes of death in U.S. special operations forces in the global war on terrorism: 2001–2004. *Ann Surg*. 2007;245:986–991.
10. Tieu BH, Holcomb JB, Schreiber MA. Coagulopathy: its pathophysiology and treatment in the injured patient. *World J Surg*. 2007;31:1055–1064.
11. Nathan HJ, Polis T. The management of temperature during hypothermic cardiopulmonary bypass: II–effect of prolonged hypothermia. *Can J Anaesth*. 1995;42:672–676.
12. Sterz F, Zeiner A, Kurkciyan I, et al. Mild resuscitative hypothermia and outcome after cardiopulmonary resuscitation. *J Neurosurg Anesthesiol*. 1996;8:88–96.
13. Danzl DF, Pozos RS, Auerbach PS, et al. Multicenter hypothermia survey. *Ann Emerg Med*. 1987;16:1042–1055.
14. Jurkovich GJ, Greiser WB, Luterman A, Curreri PW. Hypothermia in trauma victims: an ominous predictor of survival. *J Trauma*. 1987;27:1019–1024.
15. Luna GK, Maier RV, Pavlin EG, Anardi D, Copass MK, Oreskovich MR. Incidence and effect of hypothermia in seriously injured patients. *J Trauma*. 1987;27:1014–1018.
16. Peng RY, Bongard FS. Hypothermia in trauma patients. *J Am Coll Surg*. 1999;188:685–696.
17. Steinemann S, Shackford SR, Davis JW. Implications of admission hypothermia in trauma patients. *J Trauma*. 1990;30:200–202.
18. Michelson AD, Barnard MR, Khuri SF, Rohrer MJ, MacGregor H, Valeri CR. The effects of aspirin and hypothermia on platelet function in vivo. *Br J Haematol*. 1999;104:64–68.
19. Reed RL II, Johnson TD, Hudson JD, Fischer RP. The disparity between hypothermic coagulopathy and clotting studies. *J Trauma*. 1992;33:465–470.
20. Reed RL II, Bracey AW Jr, Hudson JD, Miller TA, Fischer RP. Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels. *Circ Shock*. 1990;32:142–152.
21. Rohrer MJ, Natale AM. Effect of hypothermia on the coagulation cascade. *Crit Care Med*. 1992;20:1402–1405.
22. Valeri CR, Khabbaz K, Khuri SF, et al. Effect of skin temperature on platelet function in patients undergoing extracorporeal bypass. *J Thorac Cardiovasc Surg*. 1992;104:108–116.

23. Watts DD, Trask A, Soeken K, Perdue P, Dols S, Kaufmann C. Hypothermic coagulopathy in trauma: effect of varying levels of hypothermia on enzyme speed, platelet function, and fibrinolytic activity. *J Trauma*. 1998;44:846–854.
24. Wolberg AS, Meng ZH, Monroe DM III, Hoffman M. A systematic evaluation of the effect of temperature on coagulation enzyme activity and platelet function. *J Trauma*. 2004;56:1221–1228.
25. Gubler KD, Gentilello LM, Hassantash SA, Maier RV. The impact of hypothermia on dilutional coagulopathy. *J Trauma.* 1994;36:847–851.
26. Bunker JP, Goldstein R. Coagulation during hypothermia in man. *Proc Soc Exp Biol Med*. 1958;97:199–202.
27. Rand MD, Lock JB, van't Veer C, Gaffney DP, Mann KG. Blood clotting in minimally altered whole blood. *Blood.* 1996;88:3432–3445.
28. Segel I. Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems. New York, NY: Wiley-Interscience Pub; 1993.
29. Al Dieri R, Peyvandi F, Santagostino E, et al. The thrombogram in rare inherited coagulation disorders: its relation to clinical bleeding. *Thromb Haemost*. 2002;88:576–582.
30. Valeri CR, Feingold H, Cassidy G, Ragno G, Khuri S, Altschule MD. Hypothermia-induced reversible platelet dysfunction. *Ann Surg*. 1987;205:175–181.
31. Mann KG, Brummel K, Butenas S. What is all that thrombin for? *J Thromb Haemost*. 2003;1:1504–1514.
32. Hattersley PG. Activated coagulation time of whole blood. *JAMA*. 1966;196:436–440.
33. Tripodi A, Caldwell SH, Hoffman M, Trotter JF, Sanyal AJ. Review article: the prothrombin time test as a measure of bleeding risk and prognosis in liver disease. *Aliment Pharmacol Ther*. 2007;26:141–148.
34. Brohi K, Cohen MJ, Davenport RA. Acute coagulopathy of trauma: mechanism, identification and effect. *Curr Opin Crit Care*. 2007; 13:680–685.
35. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. *J Trauma*. 2003;54:1127–1130.
36. MacLeod JB, Lynn M, McKenney MG, Cohn SM, Murtha M. Early coagulopathy predicts mortality in trauma. *J Trauma*. 2003;55: 39–44.
37. Maegele M, Lefering R, Yucel N, et al. Early coagulopathy in multiple injury: an analysis from the German trauma registry on 8724 patients. *Injury*. 2007;38:298–304.
38. Schreiber MA, Perkins J, Kiraly L, Underwood S, Wade C, Holcomb JB. Early predictors of massive transfusion in combat casualties. *J Am Coll Surg*. 2007;205:541–545.